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Chemical Mediated Synthesis of Silver Nanoparticles and its Potential Antibacterial Application

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1. Introduction

1.1 Nanometer

A nanometer is a *unit of measure* just like inches, feet and miles. By definition a nanometer is one-billionth of a meter. A meter is about 39 inches long. A billion is a thousand times bigger than a million, as a number you write it out as 1,000,000,000. That is a big number and when you divide a meter into one billion parts, well that is very small.

A nanometer is used to measure things that are very small. Atoms and molecules, the smallest pieces of everything around us, are measured in nanometers.

1.2 Nanotechnology

Nanoscience is the study, and nanotechnology is the exploitation, of the strange properties of materials smaller than 100 nanometers (nm) to create new useful objects. This work is made possible by being able to manipulate structures at the size-scale of the atoms.

Nanotechnology, or, as it is sometimes called, Engineering at the Molecular Level, is multi-disciplinary area of applied science and engineering that deals with the design and manufacture of extremely small components and systems. They are built at the molecular level of matter, are characterized by large surface areas in comparison with their volumes, and have behaviors that are governed by the laws of quantum mechanics.

Here are the few definitions of Nanotechnology.

- **Nanotechnology** is the engineering of functional systems at the molecular scale.
- **Nanotechnology** is an emerging, interdisciplinary area of research with important commercial applications, and will, most assuredly, be a dominant technology in new-world economics.
- **Nanotechnology** refers to the projected ability to construct items from the bottom up, using techniques and tools being developed today to make complete, high performance products.
- **Nanotechnology** is a field of applied science focused on the design, synthesis, characterization and application of materials and devices on the nanoscale.
- **Nanotechnology** is a subclassification of technology in colloidal science, biology, physics, chemistry and other scientific fields and involves the study of phenomena and

manipulation of material at the nanoscale, in essence an extension of existing sciences into the nanoscale.

- **Nanotechnology** addresses our ability to understand and manipulate the physical and technological characteristics that govern the behavior of a class of systems that possess at least one physical dimension that is (typically) on the order of 100 nm or less.

Two main approaches are used in nanotechnology:

- **“bottom-up” approach** where materials and devices are built up atom by atom.
- **“top-down” approach** where they are synthesized or constructed by removing existing material from larger entities.

A unique aspect of nanotechnology is the vastly increased ratio of surface area to volume present in many nanoscale materials, which opens new possibilities in surface-based science, such as catalysis.

Lithography is a top-down fabrication technique where a bulk material is reduced in size to nanoscale pattern.

In contrast, bottom-up techniques build or grow larger structures atom by atom or molecule by molecule. These techniques include Chemical synthesis, self-assembly and positional assembly.

There are three distinct nanotechnologies:

"Wet" nanotechnology is the study of biological systems that exist primarily in a water environment. The functional nanometer-scale structures of interest here are genetic material, membranes, enzymes and other cellular components. The success of this nanotechnology is amply demonstrated by the existence of living organisms whose form, function, and evolution are governed by the interactions of nanometer-scale structures.

"Dry" nanotechnology derived from surface science and physical chemistry, focuses on fabrication of structures in carbon (for example, fullerenes and nanotubes), silicon, and other inorganic materials. Unlike the "wet" technology, "dry" techniques admit use of metals and semiconductors. The active conduction electrons of these materials make them too reactive to operate in a "wet" environment, but these same electrons provide the physical properties that make "dry" nanostructures promising as electronic, magnetic, and optical devices. Another objective is to develop "dry" structures that possess some of the same attributes of the self-assembly that the wet ones exhibit.

Computational nanotechnology permits the modeling and simulation of complex nanometer-scale structures. The predictive and analytical power of computation is critical to success in nanotechnology: nature required several hundred million years to evolve a functional "wet" nanotechnology; the insight provided by computation should allow us to reduce the development time of a working "dry" nanotechnology to a few decades, and it will have a major impact on the "wet" side as well. These three nanotechnologies are highly interdependent. The major advances in each have often come from application of techniques or adaptation of information from one or both of the others.

Nanomaterials display unique, superior and indispensable properties and have attracted much attention for their distinct characteristics that are unavailable in conventional macroscopic material. Their uniqueness arises specifically from higher surface to volume ratio and increased percentage of atoms at the grain boundaries. They represent an important class of materials in the development of novel devices that can be used in various physical, biological, biomedical and pharmaceutical applications.

1.3 Silver nanoparticle

Silver is a nontoxic, safe inorganic antibacterial agent used for centuries and is capable of killing about 650 type of diseases causing microorganisms. Silver has been described as being oligodynamic because of its ability to exert a bactericidal effect at minute concentrations. It has a significant potential for a wide range of biological applications such as antifungal agent, antibacterial agents for antibiotic resistant bacteria, preventing infections, healing wounds and anti-inflammatory. Silver ions (Ag^+) and its compounds are highly toxic to microorganisms exhibiting strong biocidal effects on many species of bacteria but have a low toxicity towards animal cells. Therefore, silver ions, being antibacterial component, are employed in formulation of dental resin composites, bone cement, ion exchange fibers and coatings for medical devices.

1.4 Applications of silver nanoparticles

1.4.1 Silver as a biocide

Silver (Ag) is a transition metal element having atomic number 47 and atomic mass 107.87. Its action as an antibiotic comes from the fact that it is a non-selective toxic "biocide." Silver based antimicrobial biocides are used as wood preservatives. In water usage, silver and copper based disinfectants are used in hospital and hotel distribution systems to control infectious agents (for example, *Legionella*). Silver together with copper, is commonly used to inhibit bacterial and fungal growth in chicken farms and in post harvested cleaning of oysters.

Silver based topical dressing has been widely used as a treatment for infections in burns, open wounds and chronic ulcers. The Silver nanoparticles and Ag^+ carriers can be beneficial in delayed diabetic wound healing as diabetic wounds are affected by many secondary infections. These nanoparticles can help the diabetic patients in early wound healing with minimal scars (Mishra *et al.*, 2008). Silver nitrate is still a common antimicrobial used in the treatment of chronic wounds (Wright *et al.*, 1999).

1.4.2 Colloidal silver

Scientists have discovered that the body's most important fluids are colloidal in nature, suspended ultra-fine particles. For example blood carries nutrition and oxygen to the body cells. An electro-colloidal method (electrical silver atoms) is used for the manufacture of colloidal silver. Colloidal silver appears to be a powerful, natural antibiotic and protective against infections. Acting as a catalyst, it reportedly disables the enzyme that one-celled bacteria, viruses and fungi need for their oxygen metabolism. They suffocate without corresponding harm occurring to human enzymes or parts of the human body chemistry. The result is the destruction of disease-causing organisms in the body and in the food.

1.4.3 Silver nanoparticles as a catalyst

A possible application of silver nanoparticles is the use as a catalyst Silver was prepared by the deposition-precipitation method and was found to be a novel visible light driven photocatalyst. The catalyst showed high efficiency for the degradation of nonbiodegradable azodyes and the killing of *Escherichia coli* under visible light irradiation ($\lambda > 420 \text{ nm}$). The catalyst activity was maintained effectively after successive cyclic experiments under UV or visible light irradiation without the destruction of AgBr . On the basis of the characterization

of X-ray diffraction, X-ray photoelectron spectroscopy, and Auger electron spectroscopy, the surface Ag species mainly exist as Ag^0 in the structure of all samples before and after reaction, and Ag^0 species scavenged $h\nu_{\text{B}}^+$ and then trapped e_{CB}^- in the process of photocatalytic reaction, inhibiting the decomposition of AgBr. The studies of ESR and H_2O_2 formation revealed that $\cdot\text{OH}$ and $\text{O}_2\cdot^-$ were formed in visible light irradiated aqueous Ag/AgBr/ TiO_2 suspension, while there was no reactive oxygen species in the visible light irradiated Ag^0/TiO_2 system. The results indicate that AgBr is the main photoactive species for the destruction of azodyes and bacteria under visible light (Hu *et al.*, 2006). Silver nanoparticles immobilized on silica spheres have been tested for their ability to catalyze the reduction of dyes by sodium borohydride (NaBH_4). Catalysis of dyes was chosen because it is easy to detect a change in color when the dyes are reduced. In the absence of silver nanoparticles the sample was almost stationary showing very little or no reduction of the dyes. The reaction time showed to be strongly dependent on the concentration of silver nanoparticles. When the concentration was doubled, the reaction time was reduced to less than one third.

Silver nanoparticles act as an electron relay, aiding in the transfer of electrons from the BH_4^- ions to the dyes, and thereby causing a reduction of the dyes. BH_4^- ions are nucleophilic while dyes are electrophilic. It has been proven that nucleophilic ions can donate electron to metal particles, while an electrophilic can capture electrons from metal particles. It has been shown that BH_4^- ions and dyes are simultaneously adsorbed on the surface of silver particles, when they were present together.

Silver nanoparticles have a strong tendency to agglomerate. This reduces the surface to volume ratio and thereby the catalytic effect. Therefore a stabilizing agent is often used to prevent agglomeration. However, the agent is adsorbed on the surface of the nanoparticles, shielding them from the oxidant and reductant and thereby inhibiting the catalysis. Instead a new method for stabilizing the nanoparticles is used. The intermolecular forces which keep the nanoparticles immobilized on the silica spheres, has proven to be strong enough to prevent the particles from forming aggregates.

1.4.4 Silver nanoparticles as a bactericidal agent

Another area where silver nanoparticles have proven to be effective is in controlling and suppressing bacterial growth. There have already been developed several applications which use the bactericidal effect of silver nanoparticles. Antibacterial properties of silver are documented since 1000 B.C., when silver vessels were used to preserve water. The first scientific papers describing the medical use of silver report the prevention of eye infection in neonates in 1881 and internal antisepsis in 1901. After this, silver nitrate and silver sulfadiazine have been widely used for the treatment of superficial and deep dermal burns of wounds and for the removal of warts (Rai *et al.*, 2009). Silver's mode of action is presumed to be dependent on Ag^+ ions, which strongly inhibit bacterial growth through suppression of respiratory enzymes and electron transport components and through interference with DNA functions (Li *et al.* 2006).

Silver in a nanometric scale (less than 100 nm) has different catalytic properties compared with those attributed to the bulk form of the noble metal, like surface Plasmon resonance, large effective scattering cross section of individual silver nanoparticles, and strong toxicity to a wide range of microorganisms (Elechiguerra *et al.*, 2005). Morones *et al.* (2005) defined the antibacterial activity of silver nanoparticles in four types of Gram negative bacteria:

Escherichia coli, *Vibrio cholerae*, *Pseudomonas aeruginosa*, and *Salmonella typhi* and suggested that silver nanoparticles attach to the surface of the cell membrane and disturb its function, penetrate bacteria, and release silver ions. Other groups determined a similar antibacterial activity in Gram positive bacteria, such as *Bacillus subtilis* (Yoon *et al.*, 2008), *Staphylococcus aureus* (Shrivastava *et al.*, 2007), and *Enterococcus faecalis* (Panacek *et al.*, 2006). Silver nanoparticles have also been found to exert antibacterial activity against some drug resistant bacteria (Birla *et al.*, 2009; Inoue *et al.*, 2010).

1.5 Synthesis of silver nanoparticles

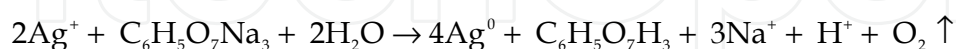
One key aspect of nanotechnology concerns the development of rapid and reliable experimental protocols for the synthesis of nanomaterials over a range of chemical compositions, sizes, high monodispersity and large scale production. A variety of techniques have been developed to synthesize metal nanoparticles, including chemical reduction using number of chemical reductants such as NaBH_4 , N_2H_4 , NH_2OH , ethanol, ethylene glycol and N, N-dimethyl formamide (DMF), aerosol technique, electrochemical or sonochemical deposition, photochemical reduction and laser irradiation technique.

Many methods, a chemical reduction method (Chou *et al.*, 2005), a polyol method (Lin & Yang, 2005) and radiolytic process (Shin *et al.*, 2004) have been developed for the synthesis of silver nanoparticles. Among the methods, chemical reduction was widely studied, due to its advantages of yielding nanoparticles without aggregation, high yield and low preparation cost (Kim *et al.*, 2004).

The chemical reduction method involves the reduction of AgNO_3 in aqueous solution by a reducing agent in the presence of a suitable stabilizer, which is necessary in protecting the growth of silver particles through aggregation. In the formation of silver nanoparticles by the chemical reduction method, the particle size and aggregation state of silver nanoparticles are affected by various parameters such as initial AgNO_3 concentration, reducing agent, AgNO_3 molar ratios and stabilizer concentration (Song *et al.*, 2009).

The silver nanoparticles were prepared by using chemical reduction method (Fang *et al.*, 2005). All solutions of reacting materials were prepared in double distilled water. In typical experiment 50ml of $1 \times 10^{-3}\text{M}$ AgNO_3 was heated to boiling. To this solution 5ml of 1% tri-sodium citrate was added drop by drop. During this process solution was mixed vigorously and heated until colour change was evident (pale brown). Then it was removed from the heating element and stirred until cooled to room temperature.

Mechanism of reaction could be expressed as follows:



The aqueous solution air dried up to 3 days and produced the dry powdered particles that were taken for further analysis.

1.6 Detection of silver nanoparticles

The first step is to determine whether silver nanoparticles are actually synthesized or not. Characterization of the nanoparticles, which examines with includes size, shape, and quantity. A number of different measurement techniques can be used for this purpose, including Atomic Force Microscopy (AFM), Scanning Electron Microscopy (SEM), Absorbance Spectroscopy and Dynamic Light Scattering (DLS).

1.7 Bactericidal effect of silver nanoparticles

The growth of unwanted bacteria has for a long time been and is still a problem for the food industry and in the medical field. Therefore, there is a need for methods to kill or slow down the growth of unwanted bacteria. An interesting alternative method is the use of metallic nanoparticles; silver nanoparticles.

In order to achieve an understanding of this effect, knowledge about the structure of bacteria is needed. In particular, the bacterial membrane and the contained proteins are of special interest, because the silver has to react with it in order to penetrate the bacteria.

Silver has for a long time been known to be toxic to a wide range of bacteria, and this has been utilized in various applications. Silver compounds are used as preservative in a variety of products and in the medical field to treat burns and infections. The bactericidal effect of silver can be divided into two groups; the reactive component being either silver ions or silver nanoparticles. Here a clear distinction between ions and particles has to be made. To clarify, silver ions are charged atoms (Ag^+) whereas silver nanoparticles are single crystals of nanosize dimensions.

In spite of the fact that the bactericidal effect of silver ions is well known and used it is still not fully understood. Experiments have shown that silver ions are able to make structural changes in the cell membrane. The membrane of bacteria contains a lot of sulfate-containing enzymes. This inactivation makes the membrane vulnerable and easier to penetrate for silver ions. Inside the cell, silver ions continue to destroy different parts of the cell by interacting with sulfate-groups, which are often located in the active site of enzymes. This interaction with the active site causes an inactivation of the enzymes.

Silver ions are also able to interact with phosphorus-groups of molecules and experiments have shown that this can have severe effects. One example is the interaction between silver ions and the backbone of DNA, which makes the bacteria unable to replicate itself or transcribe mRNA for new proteins. All these changes slow down the growth of the bacteria and finally kill it.

However, the bactericidal mechanism of silver nanoparticles on bacteria is almost unknown. It has been proposed that the effect is caused by some of the same mechanisms that caused the bactericidal effect of silver ions (Alcamo, 1997).

1.8 Mechanism of the bactericidal effect of silver nanoparticles

The silver nanoparticles which show interaction with the bacteria were all between 1 and 10 nm. The reason for this size dependency is probably a combination of the particles' ability to react with and penetrate the cell membrane and the higher surface to volume ratio of smaller particles. It is known that small (~ 5 nm) metallic particles present electronic effects, which are defined as changes in the local electronic structure of the surface. These effects enhance the reactivity of the nanoparticle surfaces. When the size of the silver nanoparticles decreases the percentage of the interacting atoms increases, and this could be the explanation to why small (1-100 nm) silver nanoparticles are able to interact with the bacteria. With regard to the ability of silver nanoparticles to penetrate the cell membrane, is it reasonable to believe that small nanoparticles are more capable of penetrating the cell membrane than larger nanoparticles (Morones *et al.*, 2005).

The morphology of the interacting silver nanoparticles have also been studied, and the majority of the silver nanoparticles were either octahedral, multiple-twinned icosahedral or decahedral in shape. Previous experiments have demonstrated that the {111} facets exhibit

high reactivity, which may explain why these types of particles are capable of interacting with bacteria. When silver nanoparticles are present in a solution they secrete a small amount of silver ions, which will have an additional contribution to the bactericidal effect of silver nanoparticles.

2. Characterization of nanoparticles

2.1 Visual inspection

The reduction of metal ions was roughly monitored by visual inspection of the solution by the method described by Fang *et al.* (2005). The conversion of the colourless reaction mixture to a brown colour clearly indicates the formation of silver nanoparticles.

2.2 UV-Vis spectroscopy

The reduction of metal ions was monitored by measuring the UV-Vis spectroscopy of the solution according to the method of Mie (1908), by the sampling of aliquots (3ml) of the aqueous component. The silver nanoparticles were measured in a wavelength ranging from 200-1100 nm. The UV-Vis spectroscopy measurements of silver nanoparticle was recorded on Shimadzu dual beam spectrophotometer (model UV-1650 PC) operated at a resolution of 1nm.

2.3 X-ray diffraction

The X-ray Diffraction patterns of silver nanoparticle were recorded according to the description of Wang (2000). Samples were air dried, powdered and used for XRD analysis. X-ray diffraction patterns were recorded in the scanning mode on an X'pert PRO PAN analytical instrument operated at 40 KV and a current of 30 mA with Cu K α radiation ($\lambda=1.5406$ Å). The diffraction intensities were recorded from 35° to 79.93°, in 2 θ angles. The diffraction intensities were compared with the standard JCPDS files. The software gave the information about the crystal structure of the particle.

The average size of the nanoparticles can be estimated using the Debye-Scherrer equation (Rau, 1962):

$$D = k\lambda / \beta \cos\theta,$$

Where D = Thickness of the nanocrystal,

k = Constant,

λ = Wavelength of X-rays,

β = Width at half maxima of (111) reflection at Bragg's angle 2θ ,

θ = Bragg angle.

The size of the silver nanoparticle was made from the line broadening of the (111) reflection using the Debye-Scherrer formula. According to the formula,

Constant (K) = 0.94

Wave length (λ) = 1.5406×10^{-10}

Full width at half maximum in radius (β) = $0.3553 \times \pi/180$

Diffraction bragg angle 2θ = 38.1759

θ = 19.088

$D = 0.94 \times 1.5406 \times 10^{-10} \times 180 / 0.3553 \times \cos 19.38 \times 3.14$

= 24.778×10^{-9}

2.4 Scanning electron microscopy

Morphology and size of the silver nanoparticle was investigated with the Scanning Electron Microscope (JSM 35 CF JEOL) in a resolution of 60Å at 15 KV, magnification of 5.0 K. The scale was about 32 mm to 3.6 µm. The size of the particle can be calculated by using the scale provided in the micrograph.

3. Antibacterial activity of silver nanoparticles

3.1 Well diffusion technique

The following human bacterial pathogens *Pseudomonas* sp., *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Shigella* sp., and *Salmonella* sp., were grown on nutrient agar plate and maintained in the nutrient agar slants at 4°C. Overnight culture in the nutrient broth was used for the present experimental study.

The antibacterial activity of silver nanoparticle was determined by agar well diffusion method. In this method, sterile Mueller - Hinton Agar plate was prepared. Bacterial pathogens used in the present experiment were spread over the agar plate using sterile cotton swab. The plates were allowed to dry and a sterile well - cutter of diameter 6.0mm was used to bore wells in the agar plates. Subsequently, a 50µl of the nanoparticle suspension was introduced into wells of the inoculated Mueller – Hinton Agar plates. The plates were allowed to stand for 1h or more for diffusion to take place and then incubated at 37°C for 24 h and measured the diameter of inhibitory zones in mm (Lee *et al.*, 2010).

3.2 Bacterial killing kinetics using nanosilver

To examine the bacterial killing kinetics in the presence of silver nanoparticles, a modified method described by Pal *et al.* (2007) was followed. Since the following pathogens *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae* showed a better activity. The human bacterial pathogens were grown in 10ml of nutrient broth supplemented with different doses of nanosilver (silver content 1, 2, 3, 4, 5 and 6 µl) at 37°C without agitation.

Killing kinetic rates and bacterial concentrations were determined by measuring the colony forming unit in the nutrient agar plates. Percentage of bacterial growth inhibition was calculated as per the equation of Shahi *et al.* (2003).

$$\text{BGI \%} = (\text{BC} - \text{BT}) \times 100 / \text{BC}$$

Where,

BGI = Bacterial Growth Inhibition

BC = Number of Bacterial Colonies in the Control

BT = Number of Bacterial Colonies in the Treatment

4. Statistical analysis

The results obtained in the present experiments were subjected to statistical analysis such as Mean, Standard Deviation and Correlation Coefficient was performed by MS Excel.

5. Observation

5.1 Visual inspection of silver nanoparticles

The appearance of a pale brown colour in solution and mirror like illumination on the walls of the Erlenmeyer flask clearly indicated the formation of silver nanoparticles in the reaction

mixture (Fig. 1). The colour of the solution was due to the excitation of surface plasmon vibrations in the silver nanoparticles. The powder form of silver nanoparticles prepared by the chemical reduction method is predicted in Fig. 2.

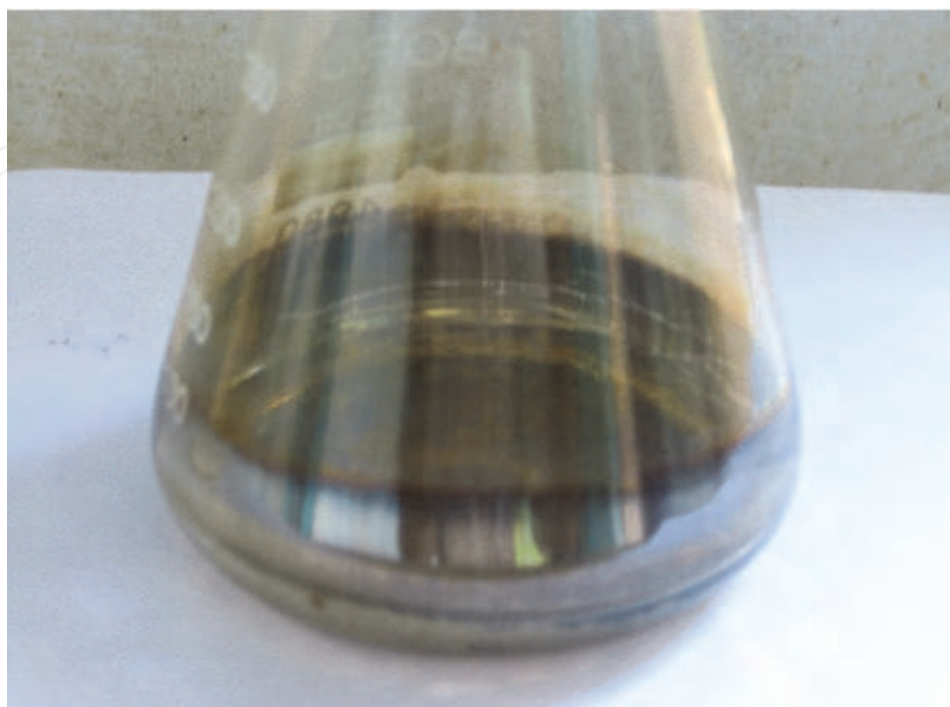


Fig. 1. The Silver Nanoparticles formed in the chemical reduction method



Fig. 2. Silver Nanoparticles in the powder form

5.2 UV-Vis spectroscopy of silver nanoparticles

The UV-Vis spectra of the silver nanoparticles showed a well defined surface plasmon band centered at around 420 nm (Fig. 3), which is the characteristic of silver nanoparticles and clearly indicate the formation of nanoparticles in solution. A minimum at ~320nm correspond to the wavelength at which the real and imaginary parts of the dielectric function of silver almost vanish. The plasmon bands are broad with an absorption tail in the longer wavelengths, which could be in practice due to the size distribution of the particle. The exact position of absorbance depends on a number of factors such as the dielectric constant of the medium, size of the particle etc.

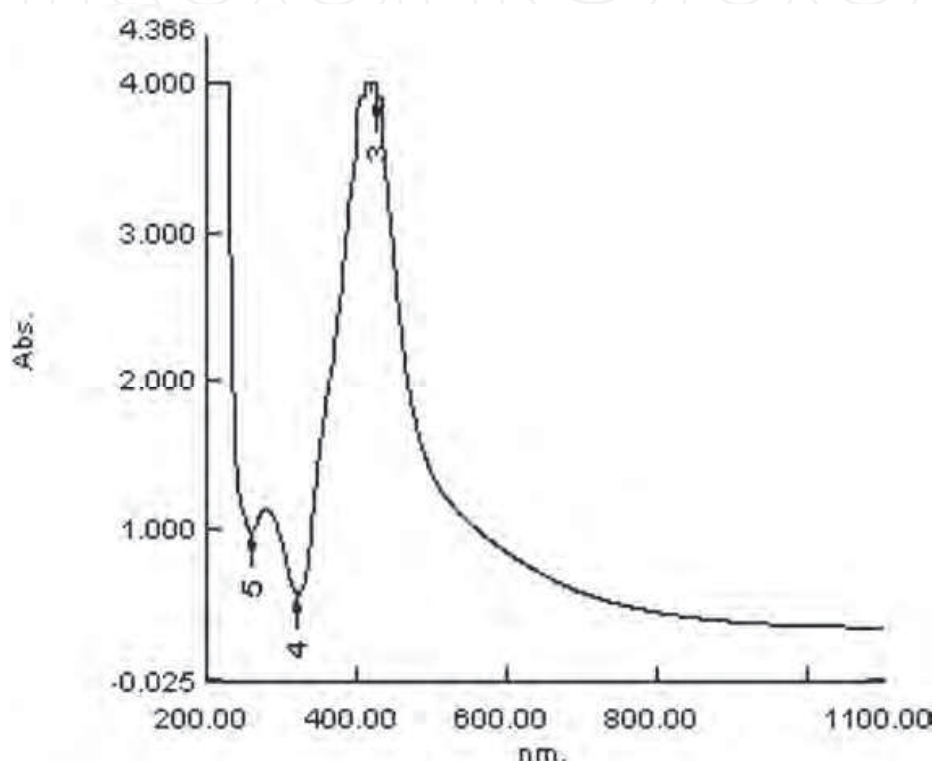


Fig. 3. UV-Vis Spectrum of Silver Nanoparticles

5.3 X-ray diffraction of silver nanoparticles

The intensive diffraction peak at a 2θ value of 38.18° from the (111) lattice plane of face centered cubic (fcc) silver unequivocally indicates that the particles are made of pure silver. Three additional broad bands are observed at 44.32° (2θ), 64.50° (2θ), and 77.05° (2θ) they correspond to the (200), (220) and (311) planes of silver respectively (Fig. 4). Other spurious diffractions are due to crystallographic impurities. Table 1 explains the X-ray diffraction peak list of silver nanoparticles. In the obtained spectrum, the Bragg peak position and their intensities were compared with the standard JCPDS files. The result shows that the particles have a cubic structure. The size of the silver nanoparticles was found to be 25 nm.

5.4 Scanning electron microscopy of silver nanoparticles

The scanning electron micrograph of silver nanoparticles is depicted in Fig. 5. The micrograph shows that the particles have a spherical nature and the average size (mean \pm SD) of the particles can be calculated as 21.22 ± 5.17 nm (Table 2).

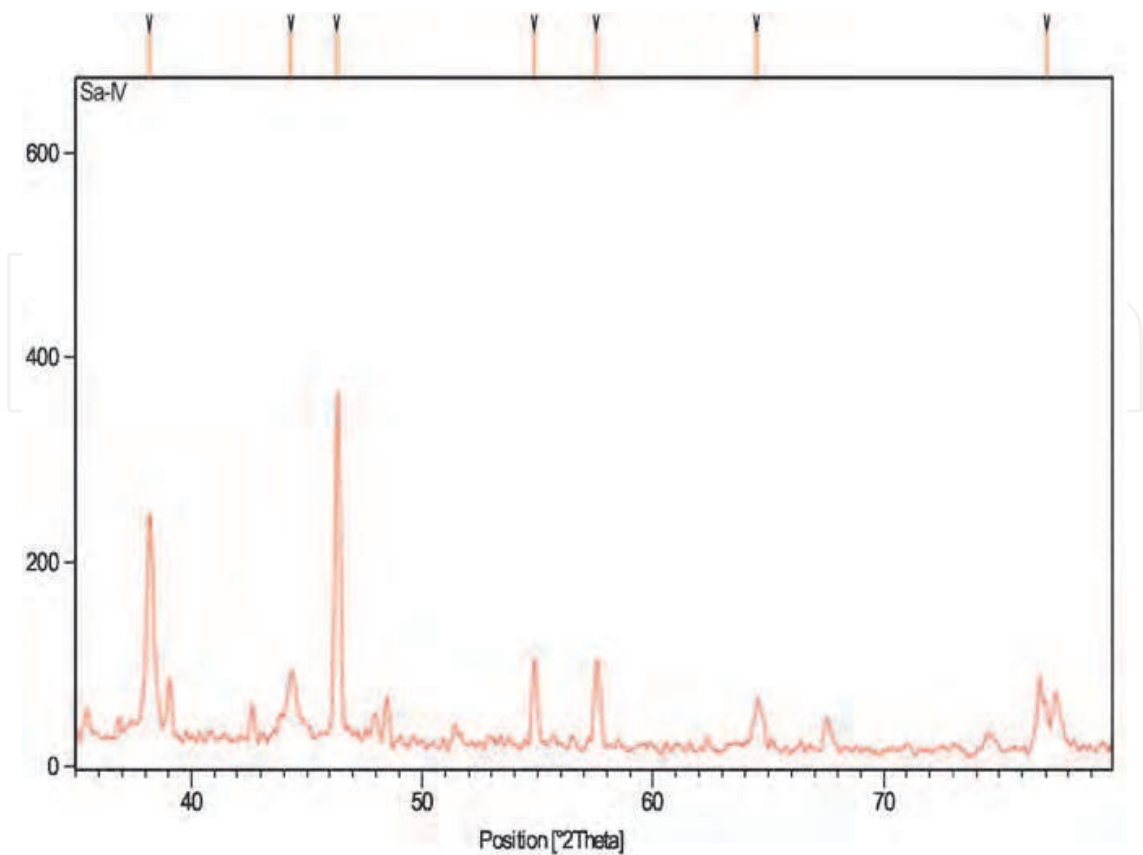


Fig. 4. X-ray Diffraction Spectrum of Silver Nanoparticles Prepared by Chemical Reduction Method

Pos. [°2Th.]	Height [cts]	FWHM [°2Th.]	d-spacing [Å]	Rel. Int. [%]
38.1759	200.85	0.3553	2.35551	31.98
44.3197	56.29	0.6764	2.04219	8.96
46.2811	338.76	0.1716	1.96011	53.95
54.8576	83.39	0.1698	1.67221	13.28
57.5224	89.84	0.1913	1.60092	14.31
64.5007	45.22	0.4181	1.44354	7.20
77.0516	55.22	0.9851	1.23670	8.79

Table 1. X-ray Diffraction Peak List of Silver Nanoparticles

5.5 Antibacterial activity of silver nanoparticles

Silver has long been considered as a powerful and natural antibiotic and antibacterial agent. Silver nanoparticles exhibit antimicrobial properties against bacterial pathogens with close attachment of the nanoparticles themselves with the microbial cells.

The antimicrobial activity of silver nanoparticle have been investigated against *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas* sp., *Shigella* sp., and *Salmonella* sp., Silver nanoparticles obtain very strong inhibitory (+++) action against *Staphylococcus aureus* followed by *Escherichia coli*, *Klebsiella pneumonia* and *Pseudomonas* sp (Table 3). After sufficient incubation the nanoparticles showed an inhibition zone near to 10mm, in, *Escherichia coli*, *Staphylococcus aureus* sp., *Klebsiella pneumoniae* and *Pseudomonas* sp (Fig.6).

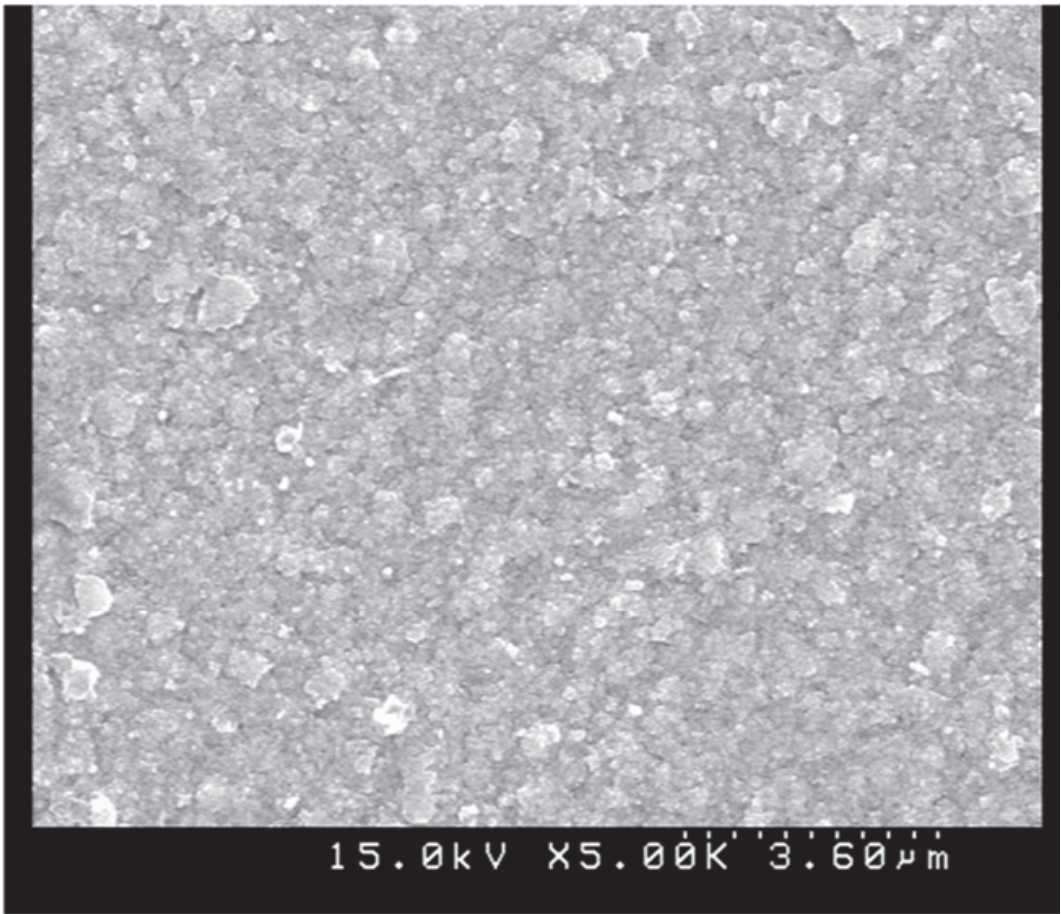


Fig. 5. Scanning Electron Micrograph of Silver Nanoparticles

S.No.	Particle size of silver (nm)
1.	28.80
2.	16.46
3.	23.04
4.	23.04
5.	11.52
6.	23.04
7.	14.40
8.	16.46
9.	23.04
10.	23.04
11.	28.80
12.	23.04
Total	254.68
Mean (Average)	21.22
Standard Deviation	± 5.17

Table 2. Size of Silver Nanoparticles Fabricated by Chemical Reduction Method

Human pathogens	Silver
<i>Escherichia coli</i>	++
<i>Staphylococcus aureus</i>	+++
<i>Klebsiella pneumoniae</i>	++
<i>Pseudomonas sp</i>	++
<i>Salmonella sp.</i> ,	-
<i>Shigella sp.</i> ,	-

+++ Very strong suppression
++ Strong suppression
-No suppression

Table 3. Antibacterial Activity of Silver Nanoparticles

5.6 Bacterial killing kinetics using nanosilver

The antibacterial properties of the colloidal silver were tested against gram positive and gram negative bacteria. The viable bacteria were monitored by counting the number of colony forming units from the appropriate dilution on nutrient agar plates. The survival fraction was determined calculating the colony forming units per milliliters of the culture. The activity was examined after 24 hrs. It was observed that the silver nanoparticles exhibit the killing rate of *Escherichia coli* to 77.86 %, *Staphylococcus aureus* to 81.8 % and *Klebsiella pneumoniae* to 70.17 % of viability. The silver nanoparticles had a better activity against *Staphylococcus aureus* (Table 4). The decrease in number of viable cells with increasing amounts of silver nanoparticles in solution can be fitted with correlation coefficient. The correlation coefficient between silver nanoparticles and selected bacterial pathogens is provided in Table 5. It revealed that there is a strong negative correlation of silver nanoparticles against selected pathogens such as *E.coli*, *Staphylococcus aureus* and *Klebsiella pneumoniae* (-0.975, -0.993 and -0.998 respectively).

Silver nanoparticle concentration (µl)	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Klebsiella pneumoniae</i>
0	280	264	295
1	220 (21.4%)	243 (7.95%)	268 (9.15%)
2	180 (35.7%)	182 (31.06%)	225 (23.7%)
3	164 (41.43%)	159 (39.77%)	186 (36.95%)
4	148 (47.14%)	126 (52.27%)	147 (50.17%)
5	124 (55.71%)	98 (62.88%)	118 (60.0%)
6	62 (77.86%)	48 (81.82%)	88 (70.17%)

Table 4. Bacterial Growth and Killing Kinetics (%) in the Presence of Nanosilver

Correlation	'r' value
Silver nanoparticle Vs <i>E.coli</i>	-0.975
Silver nanoparticle Vs <i>Staphylococcus aureus</i>	-0.993
Silver nanoparticle Vs <i>Klebsiella pneumoniae</i>	-0.998
Silver nanoparticle Vs <i>Pseudomonas Sp.</i>	-0.997

Table 5. Correlation Coefficient between Silver Nanoparticles and Selected bacterial pathogens



Fig. 6. Antimicrobial activity of silver nanoparticles against bacterial pathogens used in the experiment

6. Conclusion

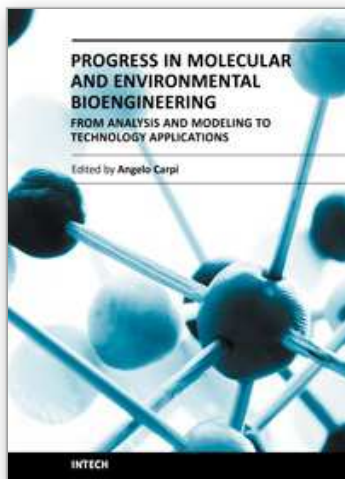
Silver nanoparticles exhibit a broad size distribution and morphologies with highly reactive facets. The major mechanism through which silver nanoparticles manifested antibacterial properties is by anchoring to and penetrating the bacterial cell wall, and modulating cellular signalling by dephosphorylating putative key peptide substrates on tyrosine residues. Silver nanoparticles act primarily in three ways against Gram-negative bacteria:

1. nanoparticles mainly in the range of 1–10 nm attach to the surface of the cell membrane and drastically disturb its proper function, like permeability and respiration;
2. they are able to penetrate inside the bacteria and cause further damage by possibly interacting with sulfur- and phosphorus-containing compounds such as DNA;
3. nanoparticles release silver ions, which have an additional contribution to the bactericidal effect of the silver nanoparticles (Feng *et al.*, 2000). Although bacterial cell lysis could be one of the reasons for the observed antibacterial property, nanoparticles also modulate the phosphotyrosine profile of putative bacterial peptides, which could thus affect bacterial signal transduction and inhibit the growth of the organisms. The effect is dose dependent and is more pronounced against gram negative organisms than gram-positive ones. The antibacterial effect of nanoparticles is independent of acquisition of resistance by the bacteria against antibiotics. However, further studies must be conducted to verify if the bacteria develop resistance towards the nanoparticles and to examine cytotoxicity (Braydich-Stolle *et al.*, 2005) of nanoparticles towards human cells before proposing their therapeutic use. Finally, this is an important area of research that deserves all our attention owing to its potential application in the fight against multi-drug resistant microorganisms.

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